Development of direct ionization methods for mass spectrometry – biomedical applications

PhD theses

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Introduction

Mass spectrometric methods have been traditionally applied for the analysis of volatile species in pharmaceutical research and clinical diagnostics. The general disadvantage of traditional mass spectrometric methods is their inability to analyze liquid phase samples (non-volatile molecular compounds), such as peptides, proteins, carbohydrates, nucleic acids, etc. This disadvantage has been gradually solved during the last 30 years, by developing spray and desorption ionization methods. Desorption ionization methods became widely used on certain, well defined areas such as proteomics, however their universal applicability has been hindered by lack of online coupling with popular chromatographic methods, and lack of appropriate sample preparation techniques.

The next important step during the evolution of the bioanalytical mass spectrometry was the appereance of the so-called direct ionization techniques. Advantages of atmospheric pressure desorption ionization method include: (1) samples are not introduced into vacuum regime of mass spectrometer, which makes analytical procedure faster and more flexible, (2) since sample does not enter vacuum, there is no need for the removal of volatile components, such as water, (3) arbitrary objects can be investigated/analyzed this way, (4) biological systems including living organisms can be investigated in an *in vivo* and *in situ* manner. During my PhD work my primary goal was to make use of these opportunities, like utilizing direct ionization mass spectrometry – particularly desorption electrospray ionization (DESI) – in high speed screening of biological samples and developing a direct ionization mass spectrometry method capable of examining living tissues.

As biological specimens represent these above mentioned two, fundamentally different sample types (biological fluids and tissue samples) my research was divided into two parallel sub-projects.

In the case of the biological fluids the clinical laboratory diagnostics is the most important application area. The recently used methods for determination of ingerdients or pharmaceuticals in biological fluids are very simple from the analytical point of view, and the potential number of the determined molecules are limited. One of the application areas of the desorption ionization techniques is high-throughput analytics, requiring the examination of a large number of sample using identical conditions in the shortest possible timeframe. As all desorption ionization techniques have virtually eliminated the cross-contamination of samples, these methods show substantial advantages for high-throughput use. There are nevertheless two major obstacles to be overcome for high-throughput applications to be

widely applicable. One is the whether desorption ionization methods could be used for quantitative analysis; the other is the surprisingly low ionization efficiency of all the methods listed above. Traditional desorption ionization techniques have a dynamic range of only 1-2 orders of magnitude, while later techniques demonstrated significantly better range (e.g. 2-4 orders of magnitude linear calibration range for DESI) allowing them to be used for quantitative analytics. This ionization efficiency, however, is still 2-4 orders of magnitude lower than those exhibited by competing (much slower) spray-ionization techniques, practically prohibiting the widespread use of these techniques not withstanding their great potential for high-throughput use. The new method, termed solid phase extraction enhanced desorption ionization (SPEEDI), was developed to overcome the intrinsically poor sensitivity of atmospheric pressure desorption ionization methods, and also for the on-line coupling of sample preparation with these analysis methods, and also for the quick and efficient conversion of liquid phase samples into confined areas of solid layer suitable for desorption ionization-MS analysis.

The generally accepted method for the biological tissue identification is histological examination, however histological methods are not designed to provide instant results. General histological procedure involves fixation, embedding, staining and sectioning, which usually takes several hours. Further problem of histology is the subjective interpretation of results. Since histological diagnosis is established based on the visual perception of morphological tissue features, there is a high pathologist-to-pathologist variance of results. These disadvantages of histology become markedly profound when tissue identification is needed during surgical intervention.

Analysis of intact tissue samples has been attempted by using various direct ionization methods (or combination of them), however it was concluded soon, that introduction of a novel approach is necessary to fully achieve our objectives. Already, at relatively early stage of my PhD work, thermal tissue evaporation has emerged as a promising solution. Since surgery widely employs these techniques in the form of electrosurgery and laser surgery, ion formation during thermal evaporation was studied in details and it was concluded that the method indeed yields considerable ion current consisting predominantly intact molecular ions of complex lipids. Newly developed rapid evaporative ionization mass spectrometry (REIMS) provides the possibility of *in vivo, in situ* mass spectrometric tissue analysis. Experimental setup for REIMS was characterized, and equipment capable of *in vivo* analysis was tested.

Applied methods

Development of the SPEEDI method

The sample preparation method is based on the adsorption of the analyte molecules on solid phase extraction (SPE) packing and the elution of the analyte onto the membrane which seals the cartridge. Thus, matrix interference is eliminated and the analyte molecules are concentrated on the confined surface of the closing frit of the SPE cartridge using hot nitrogen to evaporate the eluent. The sample is analysed with desorption electrospray ionization technique.

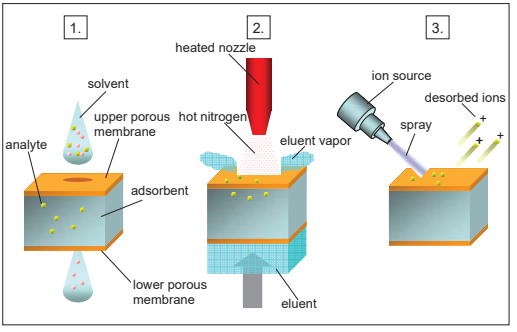

Figure 1. Scheme of the SPEEDI method.

Development of the REIMS method

Ionization of samples takes place at the surgical site, in close conjunction with the electrosurgical dissection of tissues. Electrosurgical dissection was carried out using a commercially available electrosurgical unit and a custom built electrosurgical handpiece and cutting electrode. Most important feature of cutting electrode is that the actual cutting blade is embedded into an open, stainless steel tubing. Stainless steel tubing is connected to a PTFE tubing through the handpiece. The described vent line is used for the evacuation of aerosol containing gaseous ions from the surgical site, and transmission of ions to distant mass spectrometer by Venturi gas jet pump.

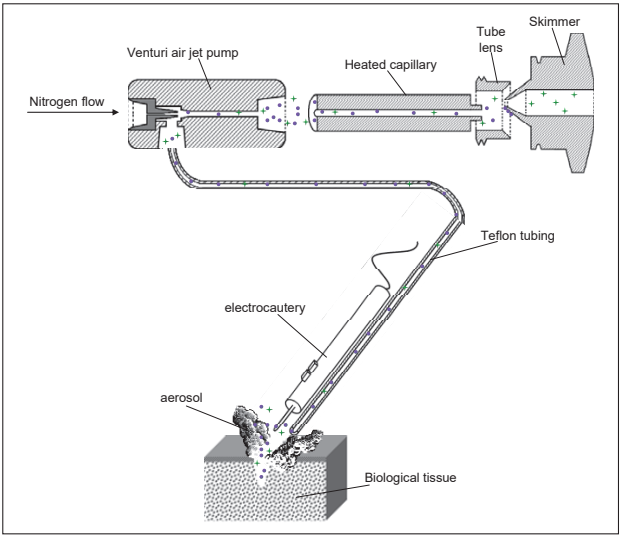

Figure 2. Scheme of the REIMS method.

Results

1. Development of a new combination of the solid phase extraction sample preparation and desorption electrospray ionization.

a) Design and construction of the sample preparation equipment and consumables needed for the technique:
   i. Special setup of SPE cartridge
   ii. SPEEDI-card
   iii. Single-channel, 96-channel elutor device and elutor for the SPEEDI-card
   iv. Heated capillary type atmospheric interface for the mass spectrometers
b) Optimization of the method using model species (Rhodamine 116).
c) Testing the applications of the method on biological fluid samples:
   i. Quantification of Cyclosporine A from urine and blood samples
   ii. Analysis of atrazine from water samples
   iii. Analysis of the ingredients of dried blood spots

2. Development of a direct ionization mass spectrometric method for *in vivo* and *in situ* tissue identification.

a) Construction of the Venturi-type interface for the ion transfer.
b) Search of a suitable mathematical method for evaluation of spectral data (principal components analysis).
c) Examination and identification of healthy tissues.
d) Identification and differentiation of healthy and cancerous tissues.

Conclusions

My PhD work was focused on the development of direct ionization methods, which are capable of the analysis of biological samples with minimal or no sample preparation.

In case of biological fluids the objective was to enhance the sensitivity and reproducibility of DESI-based methods to the level of practical applicability, while we intended to keep all the advantages of DESI regarding simplicity and speed of analysis. The described sample preparation scheme offers a simple method for concentrating the analyte content of a large volume of liquid sample into small, confined surface areas. The presented method is able to increase the sensitivity of atmospheric ionization methods to a practical level, while the unique simplicity and rapidity of the techniques are not compromised. Using this approach sensitivities similar to those of LC-MS/MS methods have been achieves, while the analysis still remained in the 2-8 s/sample range. The method can also be combined with other desorption ionization methods of choice, including MALDI, and improve the sensitivity and reproducibility of these methods.

Combination of surgical and MS techniques offers a possibility for *in situ* chemical analysis of tissue. The corresponding data was found to show high tissue-specificity, hence, a rapid statistical analysis method was developed to fully utilize this feature. It was demonstrated that (1) the overall method is fully compatible with approved surgical equipment and (2) the data evaluation algorithm is capable of differentiating malignant tumors from healthy tissue parts in real time, thus the method has real potential on the field of intraoperative tissue identification.

Publications

Júlia Dénes, Mária Katona, Ádám Hosszú, Noémi Czuczy and Zoltán Takáts, *Analysis of Biological Fluids by Direct Combination of Solid Phase Extraction and Desorption Electrospray Ionization Mass Spectrometry*, ANALYTICAL CHEMISTRY, 81(4), 1669-1675. 2009.

Karl-Christian Schäfer, Júlia Dénes, Katalin Albrecht, Tamás Szaniszló, Júlia Balog, Réka Skoumal, Dr., Mária Katona, Miklós Tóth, Dr., Lajos Balogh and Zoltán Takáts, Dr., *In Vivo, In Situ Tissue Analysis Using Rapid Evaporative Ionization Mass Spectrometry*, ANGEWANDTE CHEMIE-INTERNATIONAL EDITION, 48(44), 8240-8242. 2009.